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APPLICATION NUMBER: *60/002,368*
FILING DATE: *August 15, 1995*

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60/002368

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PROVISIONAL APPLICATION COVER SHEET

Case Docket No. VANMA28.001PRF
Date: August 15, 1995

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ATTENTION: APPLICATION BRANCH

Sir:

This is a request for filing a PROVISIONAL PATENT APPLICATION under 37 CFR § 1.53(b)(2).

For: **LIGAND OF THE ORL, RECEPTOR**

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Enclosed are:

(X) Specification in 19 pages.

(X) 7 sheets of informal drawings.

(X) A check in the amount of $150 to cover the filing fee is enclosed.

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The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Account No. 11-1410. A duplicate copy of this sheet is enclosed.

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150-114- A/Provisional

60/002368

LIGAND OF THE ORL_1 RECEPTOR.

Field of the invention.

The present invention concerns a ligand of the ORL_1 receptor, preferably a ligand which induces hyperalgesia in an animal such as a mammalian, preferably a human, and the isolated nucleic acid molecule encoding said ligand.

The present invention concerns also the inhibitor directed against said ligand or isolated nucleic acid molecule encoding said ligand.

The present invention is also related to methods for recovering said inhibitor, or antagonists and agonists of the ligand according to the invention by using the ORL_1 receptor.

Definitions.

A ligand which induces hyperalgesia in an animal, such as a mammalian, preferably a human, is a ligand which induces pro-nociceptive properties, in an animal and/or induces pain perception in an human.

An analog and/or an agonist of the peptide according to the invention, may be a molecule which mimics the peptide interaction with its receptors, especially the ORL_1 receptor, preferably a mammal ORL_1 receptor, more specifically the human ORL_1 receptor. Such may be analogs or fragments of the peptide according to the invention, or antibodies directed against ligands binding side epitopes of

the peptide receptors, or anti-idiotypic antibodies directed against particular antibodies which bind to receptor-interacting specific portions of the peptide according to the invention.

Antibodies can be raised to the peptide fragments and analogs, both in their naturally occurring forms, and in their recombinant forms. Additionally, antibodies can be raised to the peptide either in its active forms or in its inactive forms.

An antagonist of the ORL_1 receptor, preferably a mammal ORL_1 receptor, more specifically the human ORL_1 receptor, is a ligand of said receptor which blocks its interaction with the ligand according to the invention.

An inhibitor directed against the ligand according to the invention, is any molecule which may interact with said ligand in order to prevent the interaction of the ligand according to the invention with its receptors, such as the ORL_1 receptor, preferably a mammal ORL_1 receptor, more specifically the human ORL_1 receptor.

An inhibitor directed against an isolated nucleic acid molecule encoding the ligand according to the invention, is any molecule which may interact with said isolated nucleic acid molecule and prevent its expression in an animal, such as a mammalian, preferably a human.

By an endopeptide which induces hyperalgesia is meant pure animal, particularly pure mammalian, preferably pure human, endopeptide as purified from natural sources, or preferably as produced by expression in a suitable host of a recombinant DNA sequence encoding said endopeptide.

Said endopeptide may be composed of additional amino acids sequences, provided that the properties of said endopeptide as required by the present invention are maintained.

A pharmaceutical composition according to the invention, is any pharmaceutical composition comprising an effective amount of any of the products according to the

invention, and a pharmaceutically acceptable carrier.

A pharmaceutical carrier can be any compatible non-toxic substance suitable for delivering the composition of the invention to a patient.

A vector adapted for expression in a cell, preferably a mammalian cell, is any molecule or microorganism which may transfect said cell and may express in said cell the exogenous nucleic acid molecule (such as a cDNA or a genomic DNA) it comprises.

Said vector may be a plasmid, a recombinant virus, such a baculovirus, an adenovirus, ...

Description of the invention.

The ORL1 receptor is an orphan receptor whose human[1] and murine[2-8] cDNAs have been recently characterized. ORL_1 is structurally akin to opioid receptors and has been shown to be negatively coupled with adenylate cyclase[1]. ORL_1 transcripts are abundant in the central nervous system, especially in limbic areas, hypothalamus, pons and spinal cord, suggesting that the ORL_1 receptor may regulate a number of central processes including learning and memory, attention and emotions, homeostasis and sensory perception. We now report the isolation, from the rat brain, of a ligand of the ORL_1 receptor. The naturally occurring ligand was purified on the basis of its anticipated ability to inhibit forskolin-induced cAMP accumulation in stable recombinant CHO(ORL_1^+) but not in non recombinant CHO(ORL_1^-) cells. The ligand is a novel neuropeptide which resembles the endorphin dynorphin A^9 and whose amino acid sequence is Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala- Arg-Lys-Leu-Ala-Asn-Gln. The synthetic heptadecapeptide inhibits adenylate cyclase with an $IC_{50} \leq 1$ nM in CHO(ORL^+) cells in culture and, when administered in vivo, induces hyperalgesia in mice. The latter effect is consistent with the observation that in vivo inhibition of ORL_1 expression with an antisense oligonucleotide induces hypoalgesia in these animals. Taken together, our data support the notion that the newly

discovered heptadecapeptide is a potent ORL_1 receptor agonist and that it is endowed with pro-nociceptive properties.

Several laboratories have recently identified human[1] and murine[2-8] cDNAs which encode a novel G-protein-coupled receptor whose amino acid sequence is most closely related to those of opioid receptors. We[1] have named this orphan receptor ORL_1, for Opioid Receptor-Like 1. Comparison of the primary structures of ORL_1 and of mu-, delta- and kappa-opioid receptors revealed numerous amino acid identities, not only in the putative transmembrane domains but also in the four putative cytoplasmic loops. Although ORL_1 does not resemble more one subtype of opioid receptor than the two others, it displays many acidic amino acid residues in its second exofacial loop, a trait that singles the kappa out of opioid receptor types. The orphan receptor, ORL_1, mediates inhibition of forskolin-induced accumulation of cAMP by the opiate etorphine in a recombinant CHO cell line stably expressing the receptor[1]. However, etorphine is 2 to 3 orders of magnitude less potent in inhibiting the cyclase via the ORL_1 than via an opioid receptor.

ORL_1 transcripts have been detected in many discrete regions of the murine central nervous system, especially in limbic areas, hypothalamus, pons and spinal cord. Hence the hypothesis that orphan receptor ORL_1 must play a role in many central functions. Along this line, we have obtained evidence for a possible involvement of ORL_1 in the perception of pain. The strategy used was that of the antisense oligonucleotide[10] to inhibit expression of the ORL_1 receptor. Repeated in vivo treatment with an antisense oligonucleotide to ORL_1 mRNA rendered mice less reactive to thermal nociceptive stimulation. Figure 1 shows that, in the hot plate assay of Eddy and Leimbach[11], the animals which had been treated with antisense oligonucleotide mAS[25,9] displayed substantially increased latencies to rearing and escape jumping in comparison with saline-treated animals : [illegible] ($p < 0.001$) vs 20 ± 2 sec and 108 ± 6 ($p < 0.001$) vs

71 ± 6 sec, respectively. Most significantly, the "missense" oligonucleotide hAS[25,9], the human counterpart of mAS[25,9], was totally ineffective in this respect, indicating that the hypoalgesic effects elicited by the antisense mAS[25,9] were not due to non specific actions. Since a treatment with the antisense oligonucleotide should have decreased expression of the receptor, it could be predicted that ORL_1 normally facilitates pain perception.

Owing to the potential importance of this notion in neurophysiology and, possibly, neurophysiopathology, identification of an endogenous ligand of the ORL_1 receptor had become a major issue.

Our strategy for isolating an endogenous ligand of ORL1 was based on the fact that the orphan receptor is negatively coupled with adenylate cyclase. The desired compound was therefore expected to inhibit forskolin-induced accumulation of cAMP in the recombinant CHO(ORL_1^+) but not in the non recombinant CHO(ORL_1^-) cell line, as we had previously shown for etorphine (Fig. 2). The choice of the initial extraction procedure from rat brain was largely based on the structural homology of ORL_1 with opioid receptors in general and the kappa receptor in particular. Extracellular loop 2 of the kappa-opioid receptor is required for high affinity binding of dynorphins[12,13]. Since ORL_1 possesses such an acidic second exofacial loop, we surmised that the ligand in question might be a peptide which resembled dynorphin. Therefore, we used the extraction procedure that allowed Teschemacher et al. to isolate a pituitary peptide that was later identified as dynorphin A[9].

The first purification step, size exclusion chromatography of the crude peptide extract on Bio-Gel P-2, proved efficient in revealing the desired activity, i. e. inhibition of cAMP accumulation in CHO(ORL_1^+) but not in CHO(ORL_1^-) cells (Fig. 3a). The active fractions were recovered in the void volume (pool F1) and, to some extent in pool F2 (not shown), indicating that the biologically

active substance had a M_r around 1,800, the nominal exclusion limit of Bio-Gel P-2. Pools F3 to F10 were either inactive or equally effective in inhibiting (or stimulating in the case of F7) adenylate cyclase in the two CHO cell lines (data not shown). Pool F1 was further purified by cation exchange FPLC (Fig. 3b). Activity was recovered in two consecutive 1-ml fractions eluted at nearly 0.4 M NaCl, indicating the strongly basic nature of the active compound(s). These two fractions were then applied directly onto a reversed phase HPLC column and gradient-eluted with acetonitrile (Fig. 3c) to yield enough material of sufficient purity for protein sequencing. This material was found to be a heptadecapeptide of average molecular mass 1,810 and whose sequence was determined to be Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln.

To make sure that the sequence in question was not that of a major contaminant, the heptadecapeptide was synthetized as well as its ^{1}Tyr analogue for the purpose of generating a radioiodinated probe. The two peptides were obtained at ≥ 98% purity as assessed by RP-HPLC and had the predicted molecular masses (1,809 and 1,825 respectively) as assessed by mass spectrometry. Fig. 4 shows that the synthetic heptadecapeptide was very potent in inhibiting forskolin-induced accumulation of cAMP in the recombinant CHO(ORL_1^+) cell line. Its IC_{50} was 0.9 x 10^{-9} mol/l and maximal inhibition amounted to 90%. The synthetic peptide had no action on cyclase in the non recombinant CHO(ORL_1^-) cell line at concentration up to 1 µM (not shown). Interestingly, the ^{1}Tyr analogue was as effective as the parent peptide on cyclase in CHO cells expressing the receptor (IC_{50} = 1.0 x 10^{-9} mol/l, maximal inhibition > 90%) and totally inactive in the wild type cells.

The synthetic heptadecapeptide was also found to be active in vivo. Intracerebroventricular injection of 10 to 100 µg of the peptide rendered mice hyperreactive in the hot plate test. Figure 5 shows that the hyperalgesic effect

was dose dependent for 10 ng (5.5 pmol) and 100 ng (55 pmol) of peptide. A highly significant reduction of the latencies to rearing and escape jumping was observed at the larger dose of peptide: 14 ± 2 (-36%, $p < 0.01$) vs 22 ± 2 and 48 ± 2 (-26%, $p < 0.001$) vs 65 ± 3 sec, respectively. These effects were exactly the opposite of those produced by in vivo inhibition of expression of the ORL_1 receptor (Fig. 1).

The heptadecapeptide sequence did not exist in data banks although it was found to bear some resemblance with those of dynorphins, especially dynorphin A (Fig. 6). The structural homologies between this novel peptide and dynorphin A support the idea that the former will interact with the ORL_1 receptor as the latter does with the kappa-opioid receptor[12, 13, 15]. In particular, the novel peptide may be viewed as made up of a N-terminal Phe-Gly-Gly-Phe "message" for biological activity, followed by a Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys "address", for enhanced potency[15]. The "address" contains all the basic amino acid residues that are anticipated to bind the acidic second exofacial loop of the ORL_1 receptor.

The sequence of the endogenous ligand of ORL1 is likely to be highly conserved across species. Indeed, we have also isolated a bovine brain peptide with the same biological activity and the same molecular mass (1,910 ± 2) as the rat peptide. There was not enough of this peptide for complete sequencing, but 10 residues could be determined (4-8, 11, 12 and 14-16, that were identical to the corresponding amino acids in the rat sequence.

Thus, the present work has identified a novel brain peptide which is a naturally occurring, high affinity agonist of receptor ORL_1. To our knowledge this is the first example of an endogenous ligand identified from an orphan receptor. This discovery opens new avenues in neuropharmacology and may eventually find therapeutical applications. Several investigators, including ourselves, have pointed out that the ORL_1 receptor might play a crucial role in pain

perception[1,4]. Two in vivo observations now give experimental support to this notion :

(i) inhibition of receptor expression with an antisense oligonucleotide induces hypoalgesia (Fig. 1), and

(ii) intracerebroventricular administration of the heptadecapeptide induces hyperalgesia (Fig. 5), in mice.

Thus, the neuropeptide is endowed with pro-nociceptive properties and we suggest that it might be named nociceptin. Together, nociceptin and its receptor, ORL_1, may represent the molecular basis to a novel pain regulatory modality in the central nervous system.

Finally, the striking structural homologies which exist between the ORL1 and opioid receptors and between the novel peptide and endorphins, make it likely that the genes encoding the two classes of receptors and those encoding the two classes of neuropeptides have evolved in parallel, each from a common ancestor. Since dynorphin A is one of several prodynorphin[16]-derived endorphins, one may anticipate that the novel peptide is but one representative of a larger family whose other members await identification.

As the ORL_1 is located in specific parts of the brain, such as the central nervous system, especially in limbic areas, hypothalamus, pons and spinal cord, said receptor and its ligand may be involved in the control of other functions and related behaviours. For instance, the ORL_1 receptor and its ligand may regulate neuroendocrine secretion, stress, learning and memory, attention and emotions, homeostasis and sensory perception, motricity, anxiety, instinctive behaviour, ...

Thus, the present invention is related to any molecule which may affect said functions and behaviours and which is a ligand of the ORL_1 receptor.

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FIGURE LEGENDS

FIGURE 1 Effects of repeated i.c.v. injection of mouse antisense mAS[25,9]- or of human missense hAS[25,9]- oligodeoxynucleotide on the latencies to rearing and escape jumping of mice in the hot plate test of Eddy and Leimbach[11]. Each bar represents the average reaction time ± s.e.m. from 20 animals. Statistical comparisons of oligo- and saline (control)-treated groups were performed using the Student's *t* test. *** $p < 0.001$.

METHODS. The antisense oligonucleotide, mAS[25,9], was the 17-mer 5'-GGAGAAAGGACGGGGTA-3' complementary to bases 9 to 25 of the translated region of the mouse ORL_1 mRNA. The "control", missense oligonucleotide, hAS[25,9], was the 17-mer 5'-GGAGAAAGGACGAGGTA-3' complementary to bases 9 to 25 of the translated region of the human ORL_1 mRNA. The oligos were dissolved in sterile physiological saline (0.9 g/l NaCl) at the final concentration of 2 mg/ml. Male Swiss CD1 mice of 20-25 g (Charles River) were manually injected with 10 µl of solution directly into the lateral brain ventricle, on a daily basis for 4 consecutive days. Three groups of 20 mice each were used: saline-, hAS[25,9]- and mAS[25,9]-injected. The animals were tested individually, 96 hrs after the last injection, for reactivity to the thermal nociceptive stimulus.

FIGURE 2 Inhibition by etorphine of forskolin (FSK)-induced accumulation of cAMP in CHO(ORL_1^+), a recombinant CHO cell line stably expressing orphan receptor ORL_1. The CHO(ORL_1^+) line used here is a more responsive subclone of the original recombinant clone[1]. Forskolin-induced accumulation of cAMP in intact cells (2×10^5 /tube) was assayed after incorporation of [^{3}H]adenine into the intracellular pool of ATP, by selective elution of [^{3}H]cAMP from a single column of acidic alumina[1,17]. Each data point is the mean ± s.e.m. of triplicate determinations. Etorphine's IC_{50} was ~ 0.7 µM and maximum inhibition

amounted to ≈ 80%. Also shown for comparison is the effect of etorphine on FSK-induced accumulation of cAMP in non recombinant, CHO(ORL_1^-) cells.

FIGURE 3 Purification of the endogenous ligand of orphan receptor ORL_1. *a*, Size exclusion chromatography of the crude peptide extract from rat brain. Fractions were combined in 10 pools as indicated. The insert shows that the sought-after activity, inhibition of FSK-induced synthesis of cAMP in CHO(ORL_1^+) but not in CHO(ORL_1^-) cells, is present in pool F1. *b*, Cation exchange FPLC of pool F1. The molarities above the X-axis are those of NaCl in the elution gradient. A major peak of activity was eluted at about 0.4 M Nacl (fractions 67 and 68, filled rectangle). *c*, Reversed phase HPLC. The percentages above the X-axis are those of acetonitrile in the elution gradient. The desired biological activity, marked by an asterisk, was recovered as a single fraction which was eluted at 26% CH_3CN.

METHODS. *a*, Freshly dissected and rapidly frozen rat brains (35 g) were processed essentially as described by Teschemacher et al.[14] to yield a crude peptide extract. The latter was lyophilized, dissolved in 10 ml 0.1 M AcOH and loaded onto a column of Bio-Gel P-2 (2.3 x 90 cm) equilibrated in 0.1 M AcOH. Elution was with 0.1 M AcOH at room temperature. The flow rate was 0.6 ml/min. Fractions of 4 ml were collected, assayed for absorption at 280 nm and combined into pools F1 to F10 as indicated. Individual pools were lyophilized, dissolved in 2 ml phosphate buffer (20 mM $NaPO_4$, pH 6.4) and assayed for inhibition of forskolin-induced intracellular accumulation of cAMP in the CHO(ORL_1^+) and CHO(ORL_1^-) cell lines, as refered to in the legend to Fig. 1. *b*, The F1 pool (1.8 ml) was applied to a SP/8HR cation exchange column (Waters) equilibrated with 20 mM $NaPO_4$ buffer, pH 6.4 (buffer A). Elution was at 1 ml/min (about 200 psi; Waters 650E Advanced Protein purification system) with 100% buffer A for 15 min followed by a linear gradient from 100% buffer A to 50% buffer B (20 mM $NaPO_4$ + 1.0 M NaCl, pH 6.4) in 60 min. A 10-µl aliquot

of each fraction was assayed for inhibition of FSK-induced accumulation of cAMP on recombinant CHO(ORL_1^+) cells. *c*, The active fractions (67 and 68) were pooled and loaded directly onto a reversed phase HPLC column (*μ* Bondapak C_{18}, 3.9 x 300 mm; Waters). The eluents were 0.1% TFA in H_2O (eluent A) and 0.1% TFA in acetonitrile (eluent B). Elution was at a flow rate of 1 ml/min for 10 min with 100% A followed by a gradient to 10% B in 5 min and a gradient between 10% and 40% B in 50 min. Detection was at 210 nm. Fractions of 0.5 ml were collected. A 10-*μ*l aliquot of each fraction was taken to dryness (Speed Vac), redissolved in Krebs-Ringer Hepes buffer and assayed for inhibition of forskolin-induced accumulation of cAMP on recombinant CHO(ORL^+) cells. The molecular mass and sequence of the active compound in fraction 40.5 were determined with a Lasermat mass spectrometer (Finnigan MAT) and a model 476A protein sequencer (Applied Biosystems), respectively.

FIGURE 4 Inhibition by the synthetic heptadecapeptide (s-HpDPep) and its ^{1}Tyr analogue of forskolin-induced accumulation of cAMP in recombinant CHO(ORL_1^+) cells. Assay for cAMP in intact cells was as in the legend to Fig. 2. Each data point is the mean ± s.e.m. of triplicate determinations.

FIGURE 5 Effects of a single i.c.v. injection of the synthetic heptadecapeptide (s-HpDPep) on the latencies to rearing and escape jumping of mice in the hot plate assay[11]. The animals (see legend to Fig. 1) were manually injected with 0 (control), 10 or 100 ng s-HpDPep in 10 *μ*l of saline solution (NaCl, 9 g/l) and tested 20 min later. Each bar represents the average reaction time ± s.e.m. from 20 mice. Statistical comparisons of peptide- and saline (control)-treated groups were performed using the Student's *t* test. ** $P < 0.01$, *** $P < 0.001$. The experiment was repeated twice with essentially the same results

FIGURE 6 Comparison of the sequences of the endogenous heptadecapeptide refered to here as ORL_1-HpDPep, and of dynorphin. The sequence of the dynorphin is that of porcine pituitary dynorphin A^9. Identical amino acid residues are boxed. The "message" and "address" divisions of dynorphin A^{15} are indicated.

FIGURE 7 Nucleotide sequence of a partial rat brain cDNA encoding a precurseur of the nociceptine peptide. The cDNA was isolated from a lambda zap bibrary of total rat brain (stratagene) by hybridization with a probe generated by PCR on rat genomic DNA : the degenerated primers were designed from the nociceptine sequence :

A: 5'-AGATCTAGACTT$^{C}_{T}$GG$^{C}_{G}$GG$^{C}_{G}$TT$^{C}_{T}$AC$^{A\ C}_{T\ G}$GG-3'

B: 5'-CTTAAGCTT$^{T}_{C}$TG$^{A}_{G}$TT$^{A\ C}_{T\ G}$GC$^{C}_{G}$AG$^{C}_{T}$TT-3'

The underlined segments correspond to linkers comprising restriction sites for cloning.
The sequence encoding nociceptine extends fromnucleotide 364 to 414; it is flanked in 5' and 3' by codons specifying Lys-Arg dipeptides.

CLAIMS

1. Ligand of the ORL_1 receptor, preferably a mammal ORL_1 receptor, more specifically the human ORL_1 receptor.

2. Ligand according to claim 1, which induces hyperalgesia in an animal, such as a mammalian, preferably a human.

3. Ligand according to claim 1 or 2, which is a peptide, such as an endopeptide, of animal, preferably of mammalian, more specifically of human, origin.

4. Ligand according to claim 3, which is a peptide having the following amino acids sequence :
SEQ. ID NO1:
Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln,
its analogs and/or its agonists.

5. Antagonist of the ORL_1 receptor, preferably of the mammal ORL_1 receptor, more specifically the human ORL_1 receptor.

6. Antagonist according to claim 5, which is a polyclonal or monoclonal antibody or to a portion thereof.

7. Isolated nucleic acid molecule encoding the ligand of claim 3 or 4.

8. Nucleic acid molecule of claim 7, wherein the nucleic acid molecule is a DNA molecule.

9. Nucleic acid molecule of claim 8, wherein the nucleic acid molecule is a cDNA molecule.

10. Nucleic acid molecule of claim 8, wherein the nucleic acid molecule is genomic DNA.

11. Isolated nucleic acid molecule encoding the ligand of claim 3 or 4 and having at least the sequence of figure 7 or a portion thereof.

12. Inhibitor directed against the ligand according to any of the claims 1 to 4 or the isolated nucleic acid molecule according to any of the claims 7 to 11.

13. Inhibitor according to claim 12, which is a polyclonal or monoclonal antibody directed against the ligand according to any of the claims 1 to 4.

14. Inhibitor according to claim 12, which is an antisense oligonucleotide which has a sequence capable of specifically binding to the nucleic acid molecule according to any of the claims 7 to 11 so as to prevent its transcription and/or translation.

15. Inhibitor according to claim 14, comprising chemical analogs of nucleotides.

16. Inhibitor according to claim 14, said oligonucleotides having sequences which differ from one another at predefined positions.

17. Inhibitor according to any of the claims 14 to 16, wherein the oligonucleotide is coupled to a substance which inactivates the nucleic acid according to any of the claims 7 to 11.

18. Inhibitor according to claim 17, wherein said substance is a ribozyme.

19. Pharmaceutical composition comprising an element chosen among the group consisting of the ligand according to any of the claims 1 to 4, the antagonist according to the claim 5 or 6 and/or the inhibitor according to any of the claims 12 to 18, and a pharmaceutically acceptable carrier.

20. Pharmaceutical composition comprising an amount of a substance effective to reduce the expression and/or the "effects" resulting from expression of the ligand according to any of the claims 1 to 4, and a pharmaceutically acceptable carrier.

21. Pharmaceutical composition comprising an amount of a substance effective to reduce the expression and/or the "effects" resulting from expression of the isolated nucleic acid molecule according to any of the claims 7 to 11.

22. Pharmaceutical composition according to any of the claims 19 to 21, for the treatment and/or the prevention of a disease related to the following functions and/or behaviours : hyperalgesia, neuroendocrine secretion, stress, anxiety, instinctive behaviour, decreasing of learning, memory, attention and/or sensory perception.

23. Transgenic non-human animal which comprises the nucleic acid molecule according to any of the claims 7 to 11.

24. Transgenic non-human animal which comprises the nucleic acid molecule encoding the ORL_1 receptor, preferably a mammal ORL_1 receptor, more specifically a human ORL_1 receptor.

25. Method for recovering an inhibitor not known to be capable of specifically binding to a ligand according to any of the claims 1 to 4 can specifically bind to it, which comprises contacting the ligand according to any of the claims 1 to 4 under conditions permitting binding of a inhibitor known to bind the ligand according to any of the claims 1 to 4, determining the presence of any inhibitor bound to the ligand according to any of the claims 1 to 4, and recovering said inhibitor.

26. Method for recovering a compound not known to be capable of specifically binding as an antagonist or as an agonist to a ORL_1 receptor, preferably a mammal ORL_1 receptor, specifically a human ORL_1 receptor, can specifically bind to said receptor, which comprises contacting a cell, preferably a mammalian cell, comprising a vector adapted for expression in a mammalian cell, which vector further comprises nucleic acid molecule which expresses said ORL_1 receptor on the cell's surface, with the compound under conditions permitting binding of ligand known to bind to said receptor, detecting the presence of any compound bound to said receptor, and recovering said compound.

27. Method for recovering a compound not known to be capable of specifically binding as an antagonist or as an agonist to an ORL_1 receptor, preferably a mammal ORL_1 receptor, specifically a human ORL_1 receptor, can specifically bind to said receptor, which comprises preparing a cell extract from cells, preferably of mammalian cells, which comprises a vector adapted for expression in said cells, which vector further comprises nucleic acid molecule which expresses said receptor on the cell's surface, isolating a membrane fraction from the cells extract, incubating the compound with the membrane fraction under conditions permitting the binding of a ligand known to bind to said receptor, detecting the presence of any bound compound, and recovering said compound.

28. Method for recovering a compound which is not known to be capable of binding as an antagonist or as an agonist an ORL_1 receptor, preferably a mammal ORL_1 receptor, more specifically a human ORL_1 receptor, and prevent the ligand according to any of the claims 1 to 4, to activate said receptor, which comprises contacting a cell, preferably a mammalian cell, which cell comprising a vector adapted for expression in said cell, such vector further comprising nucleic acid molecule which expresses said receptor on the cell's surface with the compound under conditions permitting measure of a functional response, determining whether the compound prevents the ligand to activate said receptor, and recovering said compound.

29. Method according to claim 28, wherein the cell is a non-neuronal cell, comprising the cellular components necessary to produce a second messenger and wherein the determination of whether the compound blocks the activation of the ORL_1 receptor by a ligand according to any of the claims 1 to 4 or mimics inactivation of the ORL_1 receptor by a ligand according to any of the claims 1 to 4) comprises detecting the change in the concentration of the second messenger.

30. Method according to claim 29, wherein the second messenger is chosen among the group consisting of cyclic AMP (cAMP), inositol phosphate metabolite or intracellular calcium.

31. Method according to claim 29, wherein the modification of the second messenger is monitored by a secondary production of a report molecule (such as a luciferase, a β-galactosidase, a chloramphenicol acetyltransferase, a hormone, ...) or by the physiological modification of the cell (monitored by measure of the extra-cellular pH).

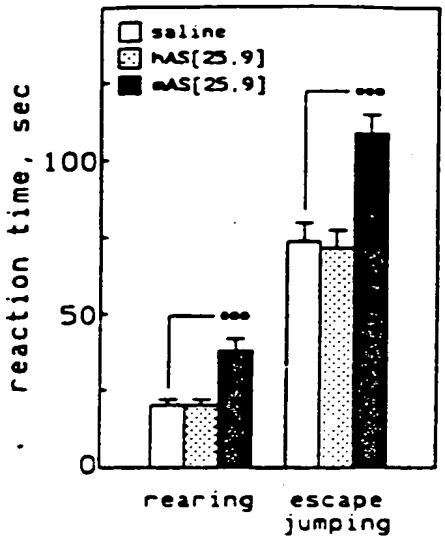
32. Method according to claim 29 to 31, wherein the non-neuronal cell is CHO.

33. Compound identified by the method according to any of the claims 25 to 31.

34. Pharmaceutical composition comprising the compound according to claim 33 and a pharmaceutically acceptable carrier.

35. Diagnostic device comprising the ligand according to any of the claims 1 to 4, an inhibitor according to any of the claims 12 to 13 and/or the ORL_1 receptor, preferably a mammal ORL_1 receptor, more specifically a human ORL_1 receptor.

36. Method of genetic treatment or prevention of a disease induced by a ligand of the ORL_1 receptor, preferably a mammal ORL_1 receptor, more specifically a human ORL_1 receptor, in an animal, specifically in a human, wherein an antagonist according to any of the claims 5 to 6 or an inhibitor according to any of the claims 12 to 18, or a nucleic acid molecule encoding said antagonist and/or said inhibitor is administered to patients with a pharmaceutically acceptable carrier to reduce the expression and/or the "activity" resulting from expression of the ligand according to any of the claims 1 to 4.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reaction time, sec
100
50
0
***
***
rearing
escape jumping

Figure 1

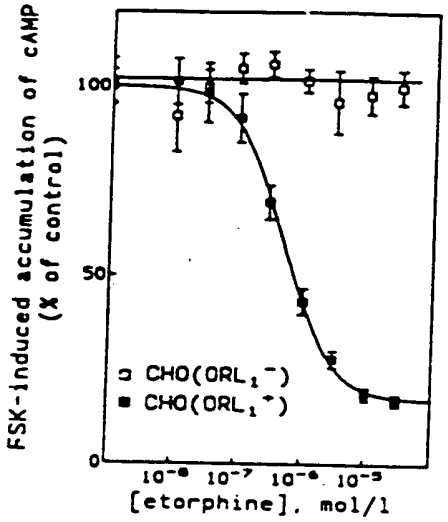

Figure 2

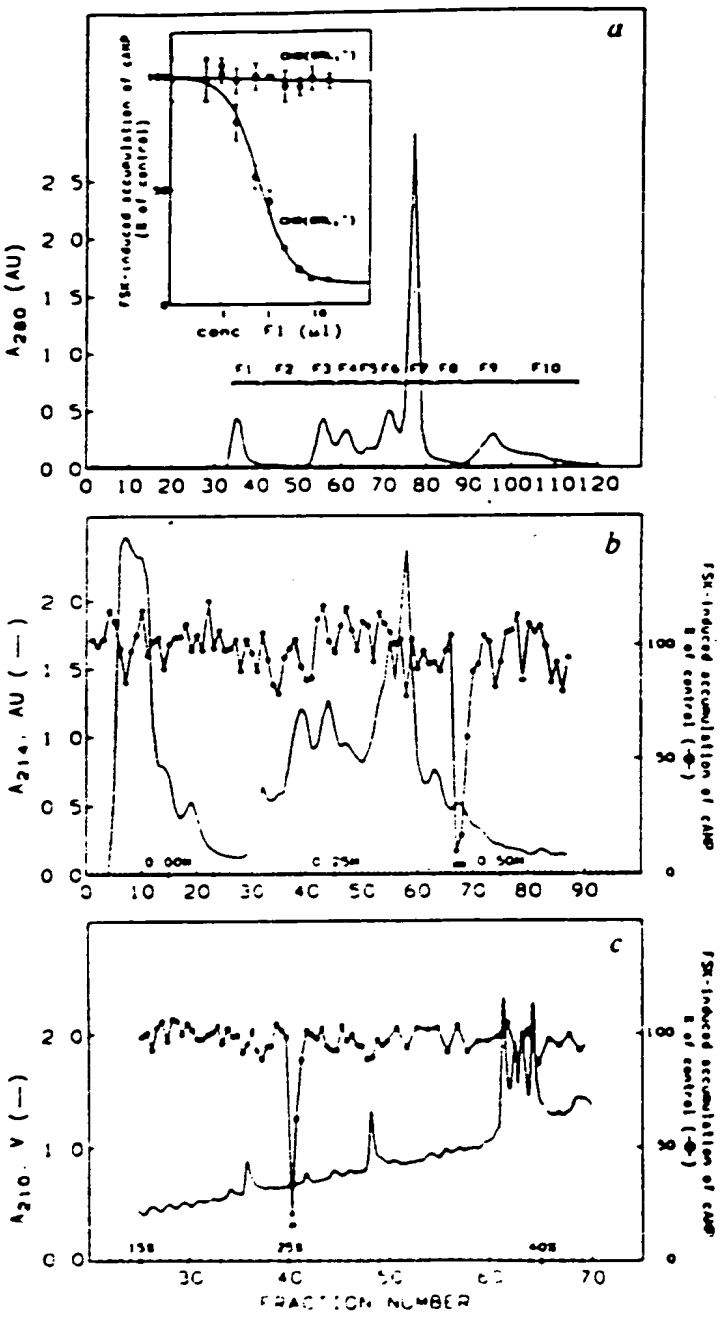

Figure 3

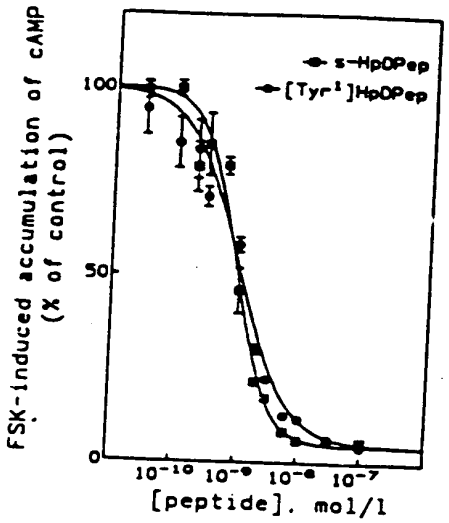

Figure 4

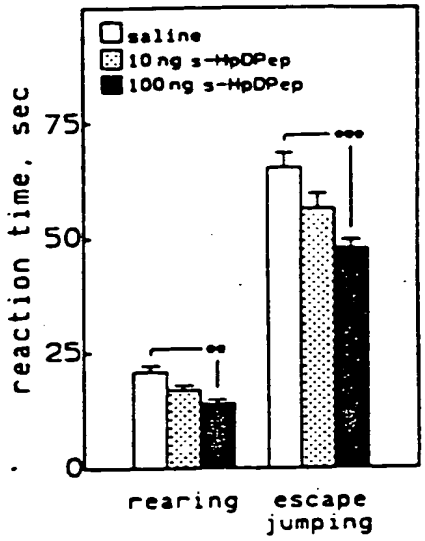

Figure 5

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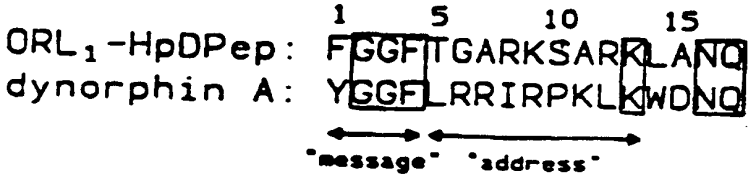

Figure 6

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          10         20         30         40         50         60
5' CTCTCCAGCGTGTTCAGCAGCTGTCCCGAGGACTGCCTCACCTGCCAGGAGAGGCTCCAC
3' GAGAGGTCGCACAAGTCGTCGACAGGGCTCCTGACGGAGTGGACGGTCCTCTCCGAGGTG
          70         80         90         100        110        120
CCGGCTCCGGGCAGCTTCAACCTGAAGCTGTGCATCCTCCAGTGTGAAGAGAAGGTCTTC
GGCCGAGGCCCGTCGAAGTTGGACTTCGACACGTAGGAGGTCACACTTCTCTTCCAGAAG
          130        140        150        160        170        180
CCCCGCCCTCTCTGGACTCTTTGCACCAAAGCCATGGCCAGTGACTCTGAGCAGCTCAGC
GGGGCGGGAGAGACCTGAGAAACGTGGTTTCGGTACCGGTCACTGAGACTCGTCGAGTCG
          190        200        210        220        230        240
CCTGCTGATCCAGAGCTCACGTCCGCTGCTCTTTACCAGTCGAAAGCCTCGGAGATGCAG
GGACGACTAGGTCTCGAGTGCAGGCGACGAGAAATGGTCAGCTTTCGGAGCCTCTACGTC
          250        260        270        280        290        300
CACCTGAAGAGAATGCCGCGTGTCAGGAGTGTGGTGCAAGCCCGAGACGCAGAGCCTGAG
GTGGACTTCTCTTACGGCGCACAGTCCTCACACCACGTTCGGGCTCTGCGTCTCGGACTC
          310        320        330        340        350        360
GCAGATGCAGAGCCTGTCGCAGATGAGGCCGATGAGGTGGAGCAGAAGCAGCTGCAGAAA
CGTCTACGTCTCGGACAGCGTCTACTCCGGCTACTCCACCTCGTCTTCGTCGACGTCTTT
          370        380        390        400        410        420
AGGTTTGGGGGCTTCACTGGGGCCCGGAAGTCAGCCCGGAAGTTGGCCAACCAGAAGCGG
TCCAAACCCCCGAAGTGACCCCGGGCCTTCAGTCGGGCCTTCAACCGGTTGGTCTTCGCC
          430        440        450        460        470        480
TTCAGTGAGTTTATGAGGCAGTACCTGGTCCTGAGCATGCAGTCAAGCCAACGCCGGCGC
AAGTCACTCAAATACTCCGTCATGGACCAGGACTCGTACGTCAGTTCGGTTGCGGCCGCG
          490        500        510        520        530        540
ACTCTGCACCAGAATGGTAATGTGTAGCCAGAAGGAGCCCCTCCCAGCTGCACCGGCCAC
TGAGACGTGGTCTTACCATTACACATCGGTCTTCCTCGGGGAGGGTCGACGTGGCCGGTG
          550        560        570        580        590        600
TGCAACCCATGAGCATCCAGGTGAGCCCCCGTACAGCATGTGTCCACACCAAGACCTGCA
ACGTTGGGTACTCGTAGGTCCACTCGGGGGCATGTCGTACACAGGTGTGGTTCTGGACGT
          610        620        630        640        650        660
GGCCGGGAGTCAGGATTCCTCCTTCCCTGAGGCACTGAACACCCGCGGCACCTCCCCACA
CCGGCCCTCAGTCCTAAGGAGGAAGGGACTCCGTGACTTGTGGGCGCCGTGGAGGGGTGT
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TGTAAAATTACCGGGGTAGAAGAACGAGTAGGAGACGGGAGAGCATCCCGGTCCACTCTC
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GAACATGAAATCAGACCTGGGGTTTTGCCTCACCACTGCCATAACTGGTTTGTAAAGGAG
CTTGTACTTTAGTCTGGACCCCAAAACGGAGTGGTGACGGTATTGACCAAACATTTCCTC
          850        860        870        880        890        900
CTGTTCTTTTTTGACTGATTGTTTGAAACAACTTTCTCCATTAAACTTCTACTGAGCAAAA
GACAAGAAAAACTGACTAACAAACTTTGTTGAAAGAGGTAATTTGAAGATGACTCGTTTT
          910        920        930
TGGTTAATAAAAAAAAAAAAAAAAAAGGAATTC 3'
ACCAATTATTTTTTTTTTTTTTTTTCCTTAAG 5'
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FIG.7